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Life-history studies of *Lydella jalisco* (Diptera: Tachinidae), a parasitoid of *Eoreuma loftini* (Lepidoptera: Pyralidae)

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Abstract. *Lydella jalisco* Woodley (Diptera: Tachinidae) is a solitary endoparasitoid of the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae), the key pest of sugarcane in Texas. This parasitoid was re-introduced into Texas in 1998 as part of a classical biological control program. Information on the biology of *L. jalisco* is scarce and indispensable for its propagation in captivity and understanding of *L. jalisco-E. loftini* interactions. Parasitoid longevity, reproductive biology and immature development were studied under laboratory conditions. At 22 °C, honey-fed mated adults lived about two weeks whereas lifespan of unmated individuals averaged three weeks. Females of *L. jalisco* emerged with a complement of eggs that continued to develop during the first days following emergence. Mating was necessary for embryonic development. After copulation, eggs were fertilized and gradually transferred to an elongated ovisac where they incubated for about one week before hatching. Hatching occurred in the female's reproductive tract, an attribute of true ovoviviparous reproduction. First instar larvae were available for oviposition during the female's entire lifetime. Egg load increased with adult female size; mean lifetime potential fecundity was 400 ± 140 eggs per female. Parasitoids successfully developed on second to sixth instar host larvae. However, host size at parasitization positively influenced the size of resultant adult parasitoids as well as the duration of the larval growth period which was shorter as host size increased. Parasitoid larvae did not complete development below 15 °C, whereas parasitoid larval and pupal mortality respectively reached 14 and 75 percent when temperature exceeded 30 °C. The importance of biological and reproductive attributes of *L. jalisco* for biological control, as well as for rearing are discussed.

Key words: Biological control, development time, fecundity, longevity, mating, parasitoid, reproductive system, size, stalkborer, temperature

Introduction

Since its accidental introduction in 1980, the Mexican rice borer (MRB), *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae) is considered to be the primary insect pest of sugarcane, *Saccharum officinarum* L., in Texas (Johnson, 1984; Legaspi et al., 1999). Attempts to control the MRB in Texas have involved cultural, chemical and host plant resistance practices, as well as biological control with a wide array of indigenous and exotic natural enemies (Legaspi et al., 1997). However, only moderate success has been achieved to date and *E. loftini* is responsible for important yield reduction each year (Legaspi et al., 1999). Furthermore, the MRB continues to expand its geographical distribution northward and threatens a variety of other suitable gramineous crops such as corn, rice, sorghum, wheat, forage and wild grasses (Johnson, 1984; Browning et al., 1989).

A promising parasitoid species was reported in Ameca, Jalisco, Mexico, parasitizing up to 33% of *E. loftini* larvae in sugarcane (Rodríguez del Bosque and Smith, 1996). This solitary endoparasitic tachinid was described as *Lydella jalisco* Woodley (Woodley, 1994). About 3,000 *L. jalisco* adults were released in sugarcane fields in the Lower Rio Grande Valley of Texas in 1989 (Pfannenstiel et al., 1990). Parasitized borers were recovered but the fly did not become established. This biological control agent was re-introduced into Texas in 1998 as part of a bi-national biological control research program (Legaspi et al., 2000). The parasitoid is being propagated at the Texas Agricultural Experiment Station (Weslaco) and its potential for reducing populations of *E. loftini* is currently being evaluated through a series of laboratory and field experiments.

A key component to a successful biological control program using *L. jalisco* will be understanding its reproductive biology. Reproductive strategies in the Tachinidae are often classified according to the type of egg, the occurrence of uterine incubation, and the method of host contact (Clausen, 1940; Belshaw, 1993). Rodríguez del Bosque and Smith (1996) indicated that females of *L. jalisco* lay incubated eggs on the host plant, probably near the entrance of borer tunnels. However, according to Retnakaran and Percy (1985) the eggs of ovoviviparous species typically hatch inside the genital duct of the female. These authors add that the term may include species that lay fully developed eggs (i.e. incubated in the ovisac) that hatch immediately after oviposition. Because stalkborer larvae are inaccessible to adult *L. jalisco* females, host contact is made by the mobile first instar larva after entry into the stalkborer tunnel. The first instar larva penetrates the host integument and develops as an endoparasitoid.

To mass rear and release the parasitoid, studies are needed to thoroughly understand the *E. loftini-L. jalisco* system. Information on the biology of the fly is largely limited to that of Rodríguez del Bosque and Smith (1996). Herein we present the results of experiments to determine the longevity of mated and unmated adults, describe the reproductive system of *L. jalisco* females, assess female parasitoid fecundity, estimate the duration of the egg incubation period, and determine how host size and temperature affect the growth and development of *L. jalisco*.

Materials and methods

Lydella jalisco *rearing*

Specimens of *L. jalisco* were initially collected in Ameca and Tala (Jalisco, Mexico) from August 1998 to March 1999 (Legaspi et al., 2000) and were used to establish a laboratory colony at the Texas Agricultural Experiment Station, Weslaco, Texas. The parasitoid has been successfully cultured since November 1998 using a technique adapted from the methods proposed by Rodríguez del Bosque and Smith (1996). Following emergence, males and females were caged together in 25 cm × 25 cm × 25 cm wood-framed screen cages to allow mating. Gender was determined by using characters of the terminalia of adults observable under a stereomicroscope. Fifteen to 20 pairs of flies were introduced into each cage, and were provided water and a 30% honey solution on a cotton ball. Environmental conditions were 22 ± 2 °C and 50 ± 10% R.H. under a 12L:12D photoperiod provided by 40 W, General Electric chroma-50 fluorescent lamps with an intensity of 30,000 lux. Humidity was slightly increased by placing a wet sponge inside each cage. Two-week-old females were dissected under a stereomicroscope, the ovisacs were removed from the abdominal cavity and ruptured in 2 ml of 0.7% formalin solution. First instar parasitoid larvae were washed for about 2 minutes, rinsed 2 or 3 times in distilled water, and immediately used for host parasitization. One or two surface-sterilized first instar parasitoid larvae and 50 µl distilled water were placed individually into 18.5 ml cups (Fill Rite, Newark, NJ), each filled with approximately 4 ml of artificial diet (Martinez et al., 1988). One fourth to fifth instar MRB larva from the laboratory colony was introduced into each cup before it was sealed with a 37.5 mm polycoated pull tab cap (Stanpac, Lewinston, NY). Parasitized borer larvae were held in an incubator at 28 ± 2 °C, 40 ± 10% R.H. in continuous darkness. Fly pupae were harvested approximately two weeks after infestation and kept in a desiccator at 22 ± 2 °C, 80 ± 5% R.H., 12L:12D until adult emergence.

Longevity of adult L. jalisco

We evaluated the longevity of adult mated and unmated *L. jalisco* males and females by taking newly emerged (< 4-hour-old) adults of similar size (n = 30) from the main colony and placing them into cages as follows: (1) males only, (2) females only, (3) males and females together (30 individuals per cage, 2 cages for treatment 3). Cages were maintained under the environmental conditions described above for adult parasitoids. Water and diluted honey were provided as food. Cages were inspected twice daily (07:00 and 17:00) and adult mortality was recorded. Monitoring continued until all parasitoids died. Mating pairs were not directly observed but at the time of their death, females from treatment 3 were dissected and mating status was recorded by assessing the content of the spermathecae at 200–400 ×. Unmated females from treatment 3, determined by the absence of spermatozoids in the spermathecae, were not used in analyses. Males from treatment 3 were assumed to have mated. The longevities of unmated versus mated males and females were compared using Student's *t*- tests.

Reproductive maturation and fecundity of L. jalisco *females*

This experiment was performed to estimate the duration of the egg incubation period (sometimes called 'gestation' period) and the potential lifetime fecundity of *L. jalisco* females. One-day-old males (n = 20) and newly emerged (< 4-hour-old) females (n = 20) were selected from the stock colony, placed into a 25 cm × 25 cm × 25 cm cage and provided water and diluted honey. Fifteen identical cages with parasitoids were prepared. To study the development of the reproductive system of *L. jalisco*, 15 females were dissected daily at ages 1–14 days and at 21 days. Five females from each of 3 cages were randomly selected for dissection. The mating status and condition of the ovaries and ovisac were recorded for each female. The number of eggs and first instar larvae were recorded separately. We measured female size with a stereomicroscope equipped with an ocular micrometer by determining the length of the left hind tibia (measured to the nearest 0.06 mm) and used this measurement as a size index. The egg loads (number of eggs and/or embryos in the ovaries and ovisac) of females of different ages were compared by ANOVA; means were separated using Tukey's HSD. Data from unmated and mated females were analyzed separately. Females with abnormal ovaries, i.e., 1.0 mm length, devoid of eggs, were eliminated from the analyses (unmated: n = 2; mated: n = 4). An analysis of covariance of egg load with female age as the covariate and body size as the continuous variable was performed using only egg load values of unmated females containing fully-developed ovaries and mated females whose eggs had not developed to first instar larvae prior

to dissection (age 4–21 days). These data provided a better estimation of age and size effects on egg load because in both cases maximum egg loads had been reached (see reproductive maturation description of *L. jalisco* in Results section). Following ANCOVA, a linear regression was performed to describe the relationship between egg load and female body size.

Effect of host size at parasitization on L. jalisco

Two hundred and forty MRB larvae of different sizes, first instar larvae excluded, were collected from the laboratory colony and weighed to the nearest 0.001 g with a precision scale (Denver Instrument Company, Arvada, CO). Host larvae (n = 190) were then manually infested with a single, surface sterilized first instar parasitoid larva. Each parasitoid larva was individually placed on the dorsum of the borer larva, near the head, using a thin brush and a stereomicroscope. Remaining borer larvae (n = 50) were used as a control. All borer larvae were individually placed into a plastic cup filled with approximately 4 ml of artificial diet and held in an incubator as previously described. Borer larvae were examined daily and the following parameters were recorded: host mortality or pupation, mortality of immature *L. jalisco*, development time (from first instar larva to adulthood), weight of the parasitoid pupa, weight of host cadaver after the parasitoid larva emerged, and the sex of each parasitoid. A logistic regression analysis was used to test the effect of host weight on the fate of the borer larva coded as 1 (parasitized borer) or 0 (unparasitized or dead borer). Infested borer larvae that died within 7 days post infestation were not included in the analysis since first instar parasitoid larvae were still too small for parasitism to be confirmed. Six borers escaped during the observation period and were also discarded. Linear regression analysis was used to estimate the relationship between host size at parasitization and the size of resultant parasitoid pupa and corresponding weight of host remains. Non linear regression analyses were used to estimate the relationship between host size and the duration of the parasitoid biological cycle.

Effect of temperature on the development of L. jalisco

Medium size host larvae (0.052 $\pm$ 0.01 g; range: 0.035 – 0.098 g; n = 250) were collected from the stock colony and individually weighed as described above. Two hundred borer larvae were manually infested with a first instar parasitoid larva as previously described. Borer larvae were individually placed into cups containing artificial diet and held in incubators at one of five selected temperatures: (1) 15:10 °C; (2) 20:15 °C; (3) 25:20 °C; (4) 30:25 °C; (5) 35:30 °C (daytime:nighttime respectively). Temperature ranges

were selected because preliminary observations indicated that parasitoids did not reach normal adulthood under constant temperatures (wing deformations were observed in adults when puparia were held at constant temperatures; I. Lauzière, unpublished data). We used 40 infested and 10 uninfested (control) borer larvae per treatment. Borer larvae were examined daily. Parasitism and parasitoid development were recorded as previously described. An ANOVA was used to compare generation times of *L. jalisco* males and females developing at different temperatures; means were separated using Tukey's HSD.

The level of significance was $\alpha = 0.05$ in all statistical analyses. The software employed for analyses was Systat 8.0 (SPSS Inc., Chicago, IL).

Results

Longevity of adult L. jalisco

For both male and female parasitoids, unmated *L. jalisco* individuals lived longer than mated ones. Unmated males lived 19.30 $\pm$ 8.28 days (mean $\pm$ SD), a week longer than mated males that lived 12.50 $\pm$ 7.43 days (Student's *t*-test, $t = 3.34$; d.f. = 29; $p < 0.01$). Similarly, unmated *L. jalisco* females lived 19.00 $\pm$ 8.67 days whereas mated females survived only 13.57 $\pm$ 8.07 days (Student's *t*-test, $t = 2.70$; d.f. =29; $p < 0.01$). The differences in longevity between sexes of similar mating status were not significant (Student's *t*-test, unmated: $t = 0.14$; d.f. = 29; $p = 0.89$; mated: $t = 0.69$; d.f. = 29; $p = 0.50$).

Reproductive maturation and fecundity of L. jalisco *females*

Females of *L. jalisco* possess 2 ovaries, each comprised of about 40–45 tightly-packed ovarioles (Figure 1). At adult emergence, the ovaries were relatively small (1.9 $\pm$ 0.2 mm $\times$ 2.2 $\pm$ 0.6 mm; n = 10). In this species, the ovaries are meroistic with polytrophic ovarioles (see Mahowald, 1972 for a review). Each ovariole held, from the tip to the base, a series of progressively enlarging follicles that were undergoing the different phases of vitellogenesis. The abdominal cavity of recently emerged females was filled with numerous large fat globules that decreased in number and size with age, becoming almost non-existent in 14- to 21-day-old females. A few days following emergence, the ovaries of unmated females occupied most of the abdominal cavity and each ovariole contained about 6 mature eggs that partially overlapped (Figure 1). The eggs of virgin females usually remained in the ovaries, although we occasionally observed resorbed eggs in the ovisac (see below). Resorbed eggs have an irregular shape, size and color.

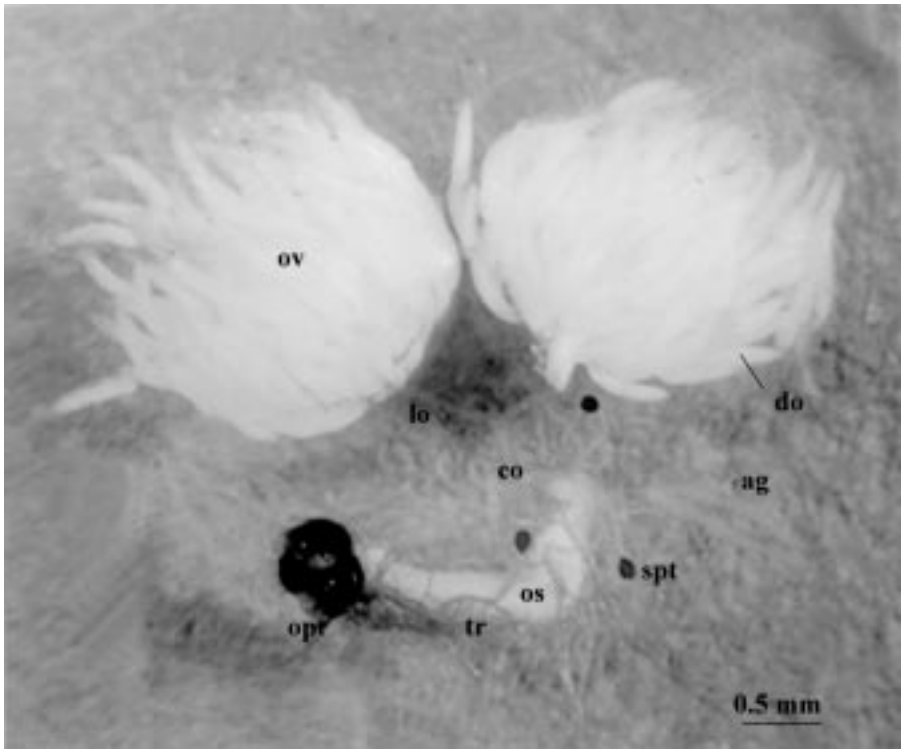

Figure 1. Structure of the reproductive system of an unmated *Lydella jalisco* female 4 days after emergence: ag, accessory gland; co, common oviduct; do, developed oocyte; lo, lateral oviduct; opt, ovipositor; os, ovisac; ov, ovary; spt, spermatheca; tr, tracheae. The reproductive organs were observed under a stereomicroscope at 30× and photographed with a Nikon Coolpix 950 digital camera. A few tracheae were removed for increased clarity.

Embryonic development never occurred in unmated females of *L. jalisco* (diploid species). After copulation, the eggs were transferred from the ovaries to the ovisac (i.e., the median oviduct developed for egg storage) through the lateral oviducts (Figure 2 A). They were fertilized prior to entering the ovisac. Sperm is stored in three spermathecae that are attached near the base of the lateral oviducts. Stored spermatozoids were observed under a microscope at 200–400× (Figure 2 B). Females apparently stored enough sperm to fertilize all eggs since spermatozoids were observed in the spermathecae of 21-day-old females, long after all the eggs were fertilized. Fertilized eggs gradually accumulated toward the base of the ovisac, arranged next to each other and uniformly orientated in a single, transverse row. Thus, the ovisac enlarged progressively over time whereas ovaries gradually shrunk and numerous tracheae became visible around the ovisac (Figure 2 A).

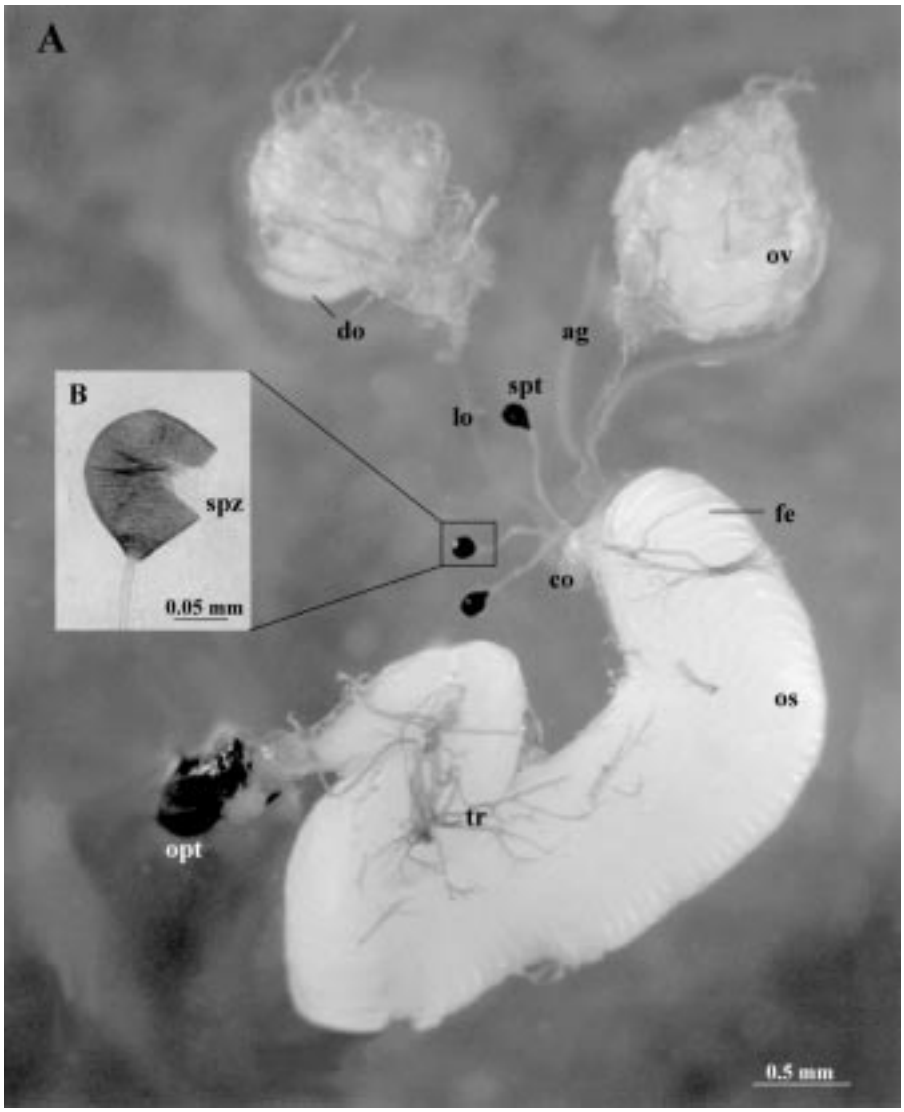

Figure 2. Structure of the reproductive system of a mated *Lydella jalisco* female 5 to 6 days after mating (A) and content of a spermatheca (B): ag, accessory gland; co, common oviduct; do, developed oocyte; fe, fertilized oocyte; lo, lateral oviduct; opt, ovipositor; os, ovisac; ov, ovary; spt, spermatheca; spz, spermatozoid; tr, tracheae. Spermathecae from the mated female were separated from the ovisac, mounted in distilled water between a microscope slide and a glass cover and observed at 400×. One spermatheca was ruptured by applying pressure and photographed.

Egg load was significantly affected by parasitoid age (ANOVA; unmated: F = 4.33; d.f. = 13, 71; $p < 0.01$; mated: F = 4.62; d.f. = 14, 118; $p < 0.01$) (Figure 3 A). Such a result is explained by the type of egg development in this parasitoid species. Females of *L. jalisco* emerged with developing eggs, some of which were too small to be accurately counted, and a few mature eggs in their ovaries. At day 1, the mean egg load of unmated females (not including eggs at the early growth phase of the oocyte) was 168.00 ± 70.89 eggs (n = 7; range 63.00–266.00) (Figure 3 A). Under our experimental conditions, most eggs reached maturity 2-3 days after adult emergence; mean total egg load was then 366.05 ± 93.41 (n = 78; range 163.00–590.00) (Figure 3 A). After the ovaries reached their full storage capacity, no further development was observed in unmated females. After 13 days, partially resorbed eggs were evident in the ovaries of unmated females. Their ovisacs occasionally contained a few (37.50 ± 19.09; n = 4) unviable (unfertilized) resorbed eggs.

Mean total egg load of mated females was 399.31 ± 139.19 eggs (n = 133; range 64.00–723.00). Soon after mating, the eggs began to accumulate in the ovisac. The ovisac of 1-day-old mated females already contained 153.14 ± 115.07 (n = 7; range 20.00–326.00) fertilized eggs (Figure 3 A). The maximum number of fertilized eggs in the ovisac was observed 5 to 6 days following emergence (492.11 ± 90.20 eggs; n = 18; range 288.00–625.00), immediately prior to egg eclosion. We refer to this as the incubation period. Its duration could not be precisely measured because embryonic development in *L. jalisco* was contingent upon successful mating and adults did not mate readily in the laboratory. Indeed, 7–30% of the 8- to 14-day-old females had mated only a few days prior to dissection, as indicated by a smaller proportion of the eggs transferred to the ovisac and partial embryonic development at the time of observation. Furthermore, only 63.1 ± 16.6% (range 29–100%) of females dissected daily were mated (Figure 3 B). High virginity rates had previously been noted in *L. jalisco* females under laboratory conditions (I. Lauzière, unpublished data). Mating appeared to occur soon after emergence of the adults as the proportion of mated females did not increase over time. Twenty one days after emergence, most females (11 of 15) left for dissection were unmated.

Egg eclosion took place in the uterus and first instar larvae were first observed in a few 7-day-old females. Egg chorions remained in the ovisac. The oviposition period could extend to the death of the female since first instar larvae were still observed on day 21. However, more first instar larvae were produced between days 9 and 14 (90.38 ± 79.22; n = 61; range 9.00–333.00). When provided hosts for oviposition, females of *L. jalisco* rarely oviposited in captivity (I. Lauzière; unpublished data). Although females from the present study were not given oviposition opportunities, both the

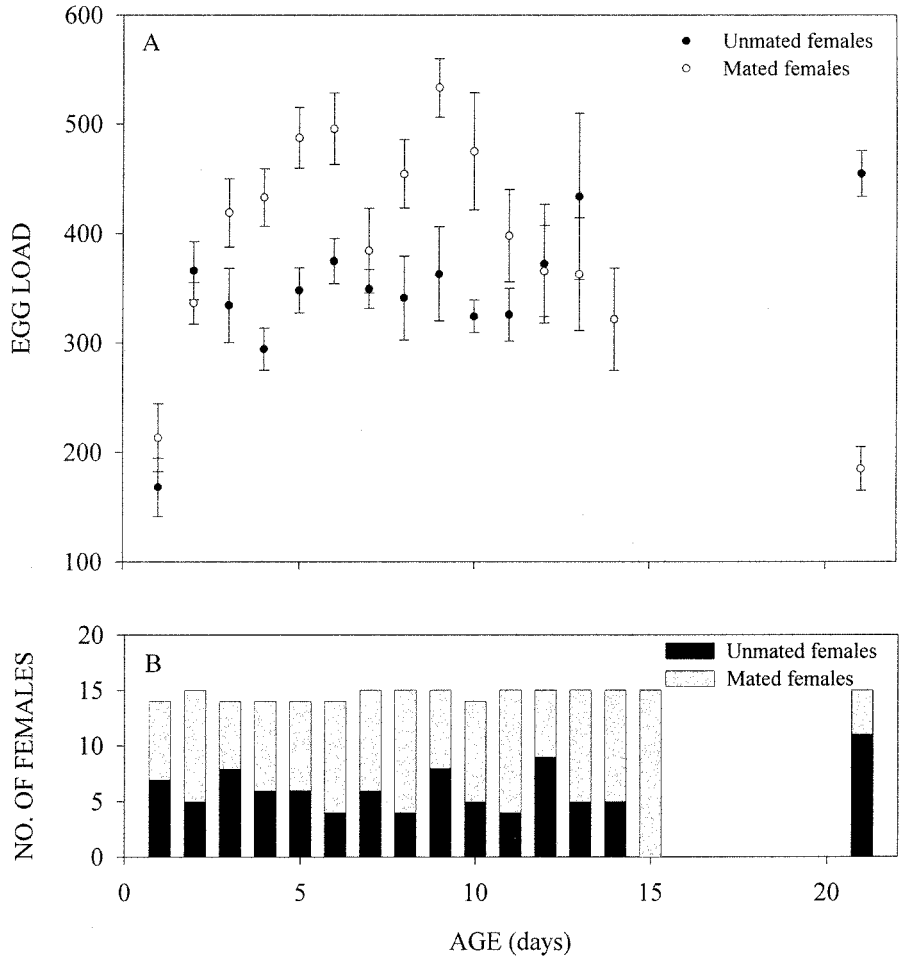

Figure 3. A. Mean (± SE) egg load (number of eggs and/or embryos in the ovaries and ovisac) of unmated and mated *Lydella jalisco* females over time. B. Total number of unmated and mated *Lydella jalisco* females at each sampling age.

number of first instar larvae and the number of developing eggs decreased after 10 days. On several occasions, first instar larvae were observed exiting the ovisac of live females through the ovipositor.

The covariance analysis of egg load (of females that had reached their maximum egg load) with female age as the covariate and body size as the continuous variable indicated that age had no effect on egg load (ANCOVA, $F = 0.13$; d.f. = 1; $p = 0.72$) whereas egg load significantly increased with increasing size (tibia length) (ANCOVA, $F = 16.98$; d.f. = 1; $p < 0.01$)

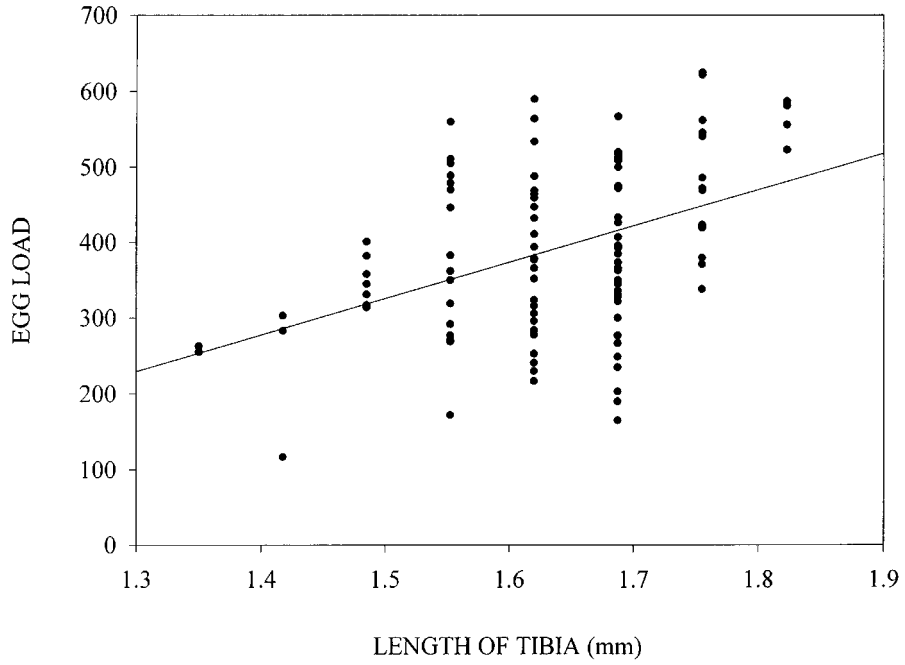

Figure 4. Egg load (number of eggs and/or embryos in ovaries and ovisac) of unmated and mated females of *Lydella jalisco* as a function of adult size. y = 481.06x – 395.77; R^2 = 0.18.

(Figure 4). Therefore, the linear regression on egg load was described by the equation y = 481.06 x – 395.77 where "x" represents the length of the tibia.

Effect of host size at parasitization on L. jalisco

Lydella jalisco first instar larvae successfully parasitized second to sixth instar MRB larvae. Parasitism was 34.2% (63 of 184) with no significant effect of host size (χ^2 = 0.28; d.f. = 1; p = 0.60). Furthermore, 27.7% (51 of 184) of the borers developed to adulthood i.e., first instar parasitoid larvae failed to penetrate the host, and 38.0% died (70 of 184). In comparison, only 8.0% (4 of 50) of uninfested borer larvae died. Most infested borers that died (61 of 70) did so within 72 hours which impeded the determination of the occurrence of parasitism, first instar larvae being too small to be found in the hemocoele. Thirty percent (19 of 63) of parasitoids died at the pupal stage, often before sexes could be determined. Observed secondary sex ratio (proportion of adult males) was 0.48. Parasitoid pupal weight varied significantly according to sexes (ANOVA, F = 12.54; d.f. = 1, 49; $p < 0.01$), male pupae being significantly smaller (0.017 ± 0.001 g; n = 25; mean ± SD) than female pupae (0.021 ± 0.001 g; n = 26).

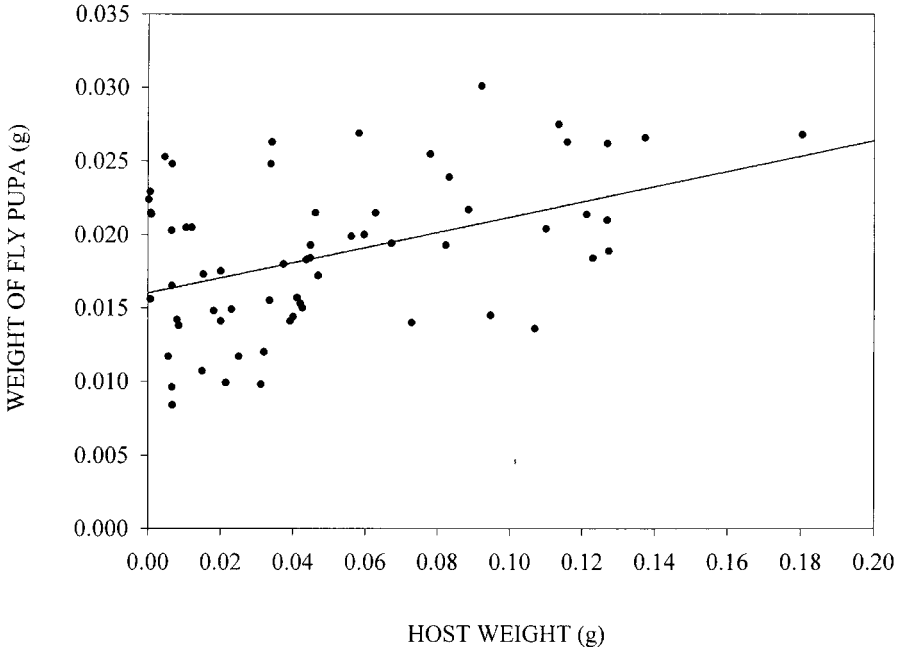

Figure 5. Weight of pupae of *Lydella jalisco* as a function of host size at parasitization. $y = 0.05x + 0.02$; $R^2 = 0.19$.

Regression analysis indicated that host size at parasitization influenced the size of resultant parasitoids and their development time. Parasitoid pupal weight showed a linear relationship to the size of the borer larva at parasitization (Figure 5). The weight of host remains also increased linearly with increasing host size at parasitization (Figure 6).

The total duration of the parasitoid development period and the duration of parasitoid larval development were expressed as a function of the size of the host in the equation $y = a + [b / (c + x)]$ where y is development time, a, b, and c are constant parameters evaluated by the model and x is the weight of the MRB larva at parasitization (Figure 7). The parasitoid pupal stage lasted 16.34 ± 2.35 days (n = 41) and did not vary according to host size (ANOVA, F = 0.17; d.f. = 1, 39; $p = 0.68$). Under laboratory conditions, the duration of the parasitoid development period from the time of infestation to adult emergence ranged from 25 to 48 days.

Effect of temperature on the development of L. jalisco

Mean total percent parasitism was 74.0% (148 of 200) and there were no significant differences between temperatures ($\chi^2 = 8.46$; d.f. = 4; $p = 0.08$).

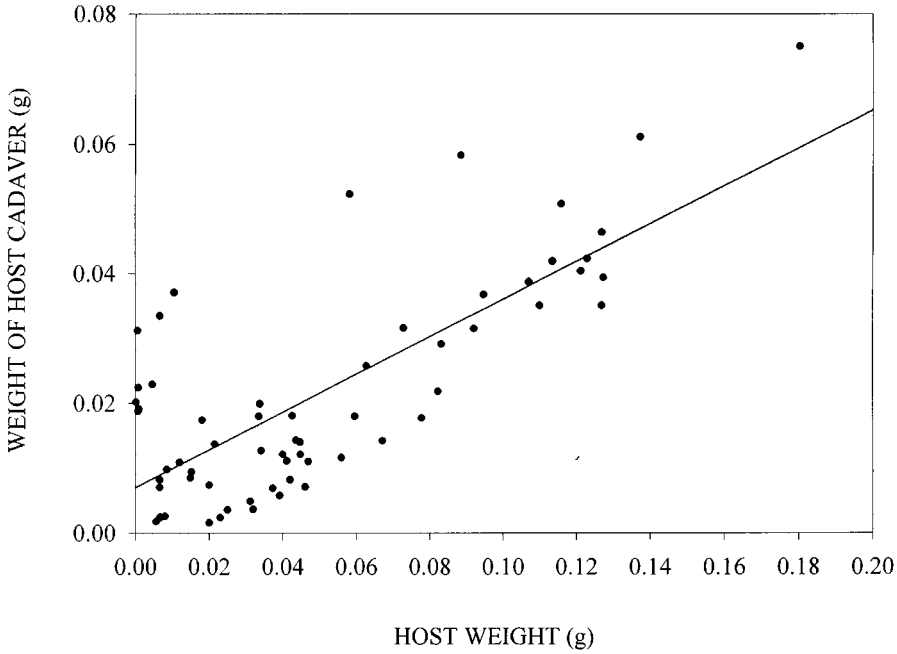

Figure 6. Weight of host cadaver after emergence of *Lydella jalisco* larvae as a function of host size at parasitization. y = 0.29x + 0.01; $R^2 = 0.58$.

In this study, 15.5% (31 of 200) of MRB developed to adulthood ('unparasitized') and the remaining 10.5% (21 of 200) of MRB larvae died. Ten percent (5 of 50) of MRB uninfested larvae died during the observation period. At the lowest temperature tested (range 15:10 °C), all medium size MRB larvae remained inactive, buried into the diet and although host larvae were large enough to support parasitoid growth and development, parasitoid larvae remained practically undeveloped. Dissections of these host larvae indicated that all 33 parasitoid larvae were still alive after 150 days and had developed about one third of the normal size reached by *L. jalisco* larvae at the time of emergence from the host. Two parasitoid larvae from treatment 2 (20:15 °C) exhibited a similar development. Parasitoid larval and pupal mortality differed significantly according to temperature (χ^2 = 14.81; d.f. = 3; $p < 0.01$). Under treatments 2, 3 and 4, larval and pupal mortality did not exceed 6.5% and 48.4%, respectively. However, at the highest temperature (treatment 5) larval mortality reached 14.3% (3 of 21) and pupal mortality 76.2% (16 of 21), resulting in only 9.5% (2 of 21) adult parasitoid emergence.

Temperature significantly decreased development time of *L. jalisco* (ANOVA, F = 20.11; d.f. = 3, 41; $p < 0.01$) (Table 1). However, the generation

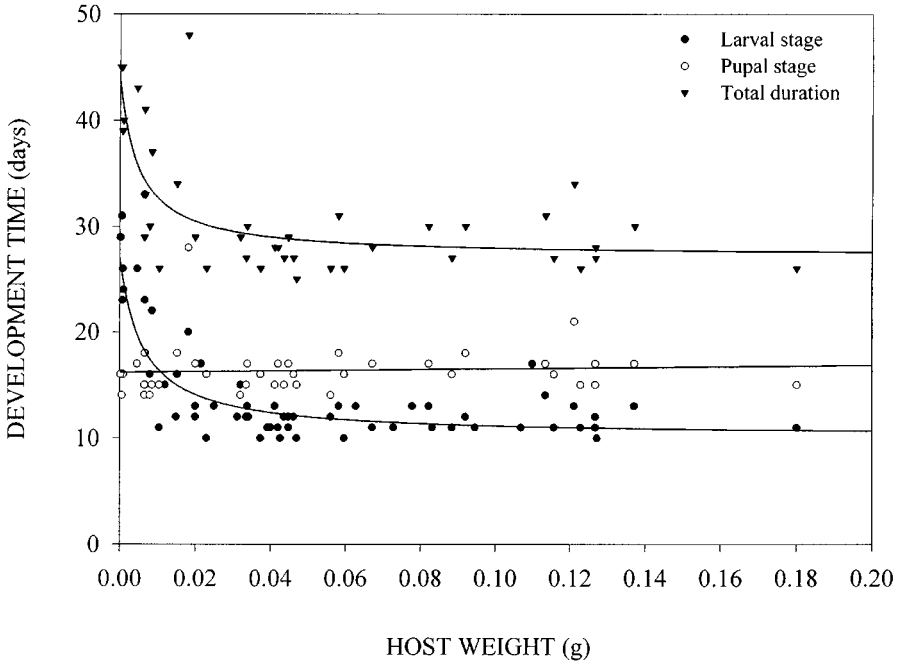

Figure 7. Development time of *Lydella jalisco* as a function of host size at parasitization. Larval stage: y = 10.19 + [0.11 / (0.006 + x)]; R^2 = 0.96 (parameter standard error values = 0.38, 0.018, 0.001, respectively). Pupal stage: y = 3.28x + 16.18; R^2 = 0.004. Total duration: y = 27.17 + [0.08 / (0.005 + x)]; R^2 = 0.98 (parameter standard error values = 0.65, 0.026, 0.002, respectively).

times of male and female parasitoids did not differ (ANOVA, sex: F = 0.40; d.f. = 1; p = 0.53; temperature × sex: F = 0.32; d.f. = 3; p = 0.81).

Discussion

The longevity of *L. jalisco* adults was affected by their mating status. Males and females caged together lived for about 13 days, whereas unmated flies lived about one week longer. These results are similar to those reported in the literature where insects were prevented from mating and lifespan was compared with that of mated individuals (i.e. Bell and Koufopanou, 1985). Differences in longevity could be attributed to energy costs associated with reproductive activities. In males, energy is expended in sperm production, courtship, and copulation, whereas in females, biological resources are channeled into maintenance of viable sperm in the spermathecae, oogenesis and embryogenesis. Although mating has not been thoroughly studied in

Table 1. Duration (days ± SD) of development period of *Lydella jalisco* at different temperatures

Temperature range (°C)	N	Larval development	N	Pupal development	N	Total
15:10*	33	> 150	–	–	–	–
20:15	31	43.5 ± 29.1b	20	29.5 ± 1.2c	20	71.2 ± 28.4b
25:20	29	20.9 ± 3.8a	13	16.1 ± 1.0b	13	36.3 ± 2.6a
30:25	28	12.1 ± 2.2a	14	10.9 ± 0.7a	14	22.1 ± 1.5a
35:30	18	11.4 ± 1.5a	2	11.0 ± 0.00a	2	20.5 ± 0.7a

Means within a column followed by the same letter are not significantly different (Tukey's HSD, $p > 0.05$).
*Treatment excluded from analysis.

L. jalisco, an extended lifespan is likely to increase mating opportunities and chances of successful progeny production.

Our results indicated that parasitoid larvae suitable for parasitization (first instar larvae) are available one week after copulation, with peak production 9–14 days after adult emergence. The occurrence of partially resorbed eggs in older, unmated females suggests that they have the ability to recycle some of the nutrients allocated to eggs through oosorption with a corresponding gain in longevity. Females of several hymenopterous and dipterous parasitoid species can resorb unlaid eggs. According to Bell and Bohm (1975), oocytes degenerate in response to behavioral, ecological or physiological factors such as food or host deprivation, virginity, and age. Energy and materials contained in those eggs can then be used both for adult maintenance and future egg production (Engelmann, 1970; Bell and Bohm, 1975; Jervis and Kidd, 1986; Jervis and Copland, 1996).

Observations of the reproductive system of *L. jalisco* indicated that females emerge with a complement of mature eggs in their ovaries (autogeny) but mating is a prerequisite for embryogenesis. Furthermore, the eggs of *L. jalisco* are incubated in an ovisac as in other tachinid species (Wood, 1987; Belshaw, 1993). Egg hatching occurs internally, matching the definition of typical ovoviviparous reproduction (Retnakaran and Percy, 1985). However, this observation does not exclude the possibility that hatching could have occurred inside the ovisac because under experimental conditions, female parasitoids did not have oviposition opportunities and carried their eggs until they hatched. Under natural conditions, *L. jalisco* may lay incubated eggs from which first instar larvae hatch soon after oviposition, a behavior most authors designate as ovolarviposition.

Although the eggs contained in the ovisac of gravid females are found at different stages of embryonic development and are not all suitable for rearing purposes, the production of first instar parasitoid larvae does represent an interesting characteristic of this biological control agent in terms of mass production because they can be relatively easily collected to infest host larvae. Furthermore, compared with other tachinid species, *L. jalisco* exhibited an intermediate fecundity sensu Clausen (1940) as mated females may produce as many as 700 eggs. Differences in fecundity across tachinid parasitoid species have been attributed to host location, host contact and host attack strategies, presumably to compensate for immature parasitoid mortality (Clausen, 1940). Thus, higher parasitoid egg production has been associated with reproductive strategies that have lower overall immature survival rates, and vice versa (Clausen, 1940; Price, 1975; Belshaw, 1994). According to Clausen (1940), species that oviposit in the immediate vicinity of the host such as *L. jalisco* have an intermediate egg production (500–1,000 eggs/female) compared with species that lay eggs in the host habitat (2,000–10,000 eggs/female) and species that oviposit on/in the host (100–200 eggs/female).

The egg load of *L. jalisco* increased linearly with female size. A positive correlation between female size and fecundity has been recorded in many other parasitoid species (i.e. Godfray, 1994; Jervis and Copland, 1996) including the Tachnidae (Bartlett, 1941). In the case of *L. jalisco*, this pattern highlights the importance of using large hosts for rearing the parasitoid since host and parasitoid sizes are positively correlated, i.e., larger hosts yielded larger parasitoids. Indeed, data indicated that all larval instars of *E. loftini* are suitable for successful growth and development of *L. jalisco*. Because adult females of *L. jalisco* do not directly contact the concealed host, a broad range of host sizes was expected to be similarly acceptable for parasitism. Nevertheless, the size of the MRB larva at parasitization affected the size of the resultant parasitoid. Furthermore, host size at parasitization affected the duration of the parasitoid development time: larval growth was delayed in small MRB larvae. *Lydella jalisco* immature larvae allowed small MRB larvae to feed and complete a few additional molts before the host was actually paralyzed and killed. *Lydella jalisco* larvae also prevented metamorphosis in large MRB larvae. As pointed out by Rodríguez del Bosque and Smith (1996), parasitized borers seem ‘normal’ until the third instar *L. jalisco* larva occupied almost the entire hemocoel and paralyzed the borer prior to exiting the host cadaver. There was apparently, however, a compromise between the size the parasitoid could reach (availability of an adequate amount of nutrients to complete its development) and the amount of time necessary to complete its development because smaller borers did yield smaller parasitoids. Further

experiments would be needed to determine physiological aspects of the relationship between *L. jalisco* and *E. loftini* larvae in early stages of the host (such as reduced larval parasitoid rate of development, quiescence, or diapause in the first instar larva; e.g. Grenier and Delobel, 1984; Mellini, 1986; Belshaw, 1994). The ability of *L. jalisco* to successfully parasitize MRB larvae that are initially too small to support parasitoid development represents another favorable attribute of this parasitoid since releases made early in the sugarcane growing season may eliminate MRB larvae before they further damage the crop. Brewer and King (1978) reported that larval growth and food consumption of the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidopotera: Pyralidae), were reduced in borers parasitized by the tachinid *Lixophaga diatraeae* (Townsend), these reductions being greater the earlier the stage of the borer. They also indicated that development time of larvae of *L. diatraeae* decreased with increasing host larva age.

Temperature was also shown to affect development time in *L. jalisco*: as temperature increased both larval and pupal developmental time decreased. Our results suggest that *L. jalisco* can continue developing under low temperatures and may overwinter within the larval stage of the host. The highest temperature tested (40:35 °C) produced the highest mortalities in larvae (14%) and pupae (76%). Unfavorable relative humidity percentages may partially explain these high rates of mortality.

There have been numerous attempts worldwide to control economically important lepidopteran pests of gramineous crops using tachinid parasitoids (Bennett, 1969; Grenier, 1988). Efforts have been particularly directed against the sugarcane borer, *D. saccharalis*, and the sugarcane internode borer, *Chilo sacchariphagus* (Bojer), both (Lepidoptera: Pyralidae), using *Lixophaga diatraeae* and *Diatraeophaga striatalis* (Townsend) (Betbeder-Matibet, 1967; Bennett, 1969). These tachinids are gregarious and exhibit a similar host attack strategy as *L. jalisco*. However, the fecundity of *L. diatraeae* is 100–150 eggs/female (Étienne, 1973) whereas that of *D. striatalis* is 200–300 eggs (Betbeder-Matibet, 1967), both considerably less than *L. jalisco*. Both species have a broad host range: fifteen host species have been reported for *L. diatraeae* and seven for *D. striatalis* (Bennett, 1969). Overall, field releases have shown inconsistent results at different sites of introduction. Failures of parasitoids to colonize have been attributed to inadequate host availability and suitability, deficient release strategies in terms of number of adult parasitoids released, periodicity, timing, and adverse environmental factors. Such failures emphasize the need for studies of the biology and ecology of those biocontrol agents.

Attempts to rear *L. jalisco* resulted in successful adaptation to confinement in laboratory cages. Although mating success was variable (Figure 3), the

parasitoid possesses several biological attributes favorable for mass rearing, including relatively high fecundity. Furthermore, egg incubation and hatching in the ovisac allow viable larvae to be easily collected, and large numbers of flies to be produced using a relatively simple rearing technique. Percentage parasitism can be increased under laboratory conditions by increasing the number of first instar parasitoid larvae per host at the time of infestation. In addition, *L. jalisco* may be specific to *E. loftini* (Rodríguez del Bosque and Smith, 1996). In the Lower Rio Grande Valley of Texas, this stalkborer does not exhibit winter diapause and is available for parasitism all year long, with populations increasing in sugarcane between June and September (Johnson, 1985; Meagher et al., 1996). However, during those months, climatic conditions in the Lower Rio Grande Valley of Texas are significantly different from those in the parasitoid's natural habitat (Legaspi et al., 2000). Field studies are underway to assess survival, parasitism and dispersal of *L. jalisco* and its efficacy against *E. loftini* on several gramineous crops including sugarcane, sorghum, corn, and rice. The combination of laboratory and field studies should allow more realistic assessments of the viability of mass rearing and parasitoid augmentation.

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